PB82-127887

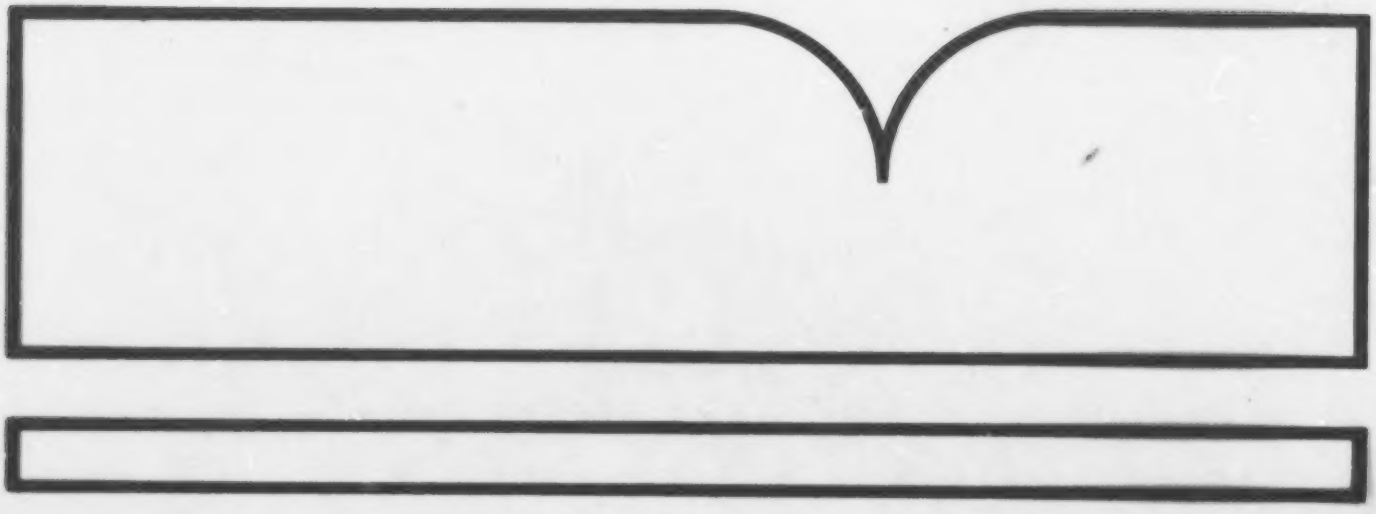
On the Role of Metallothionein in Cadmium Absorption by Rat Jejunum In situ

Cincinnati Univ., OH

Prepared for

Health Effects Research Lab.
Cincinnati, OH

7 Nov 79



U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECI
EPA-600/J-79-143	Journal ARticle	P882-127887

4. TITLE AND SUBTITLE	5. REPORT DATE
On the Role of Metallothionein in Cadmium Absorption by Rat Jejunum in situ	October 1981
	6. PERFORMING ORGANIZATION CODE 0308061
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
D. Kello, N. Sugawara, C. Voner, E.C. Foulkes	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Depts. of Environ. Health & Physiology Univ. of Cincinnati, College of Medicine Cincinnati, OH 45267	11. CONTRACT/GRANT NO. R805840010
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. EPA Health Effects Research Laboratory 26 W. St. Clair St. Cincinnati, OH 45268	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
Published in: Toxicology 14:199-208 (1979)

16. ABSTRACT
The role of metallothionein (MT) in the mechanism of cadmium absorption from the jejunum was studied in 7-9-week-old-male rats exposed to 50 ppm of cadmium in drinking water for 9 days. Exposed animals contained an average of 144 ug MT/g of mucosal tissue, compared to 40 ug in control animals. During jejunal perfusion in situ with 5 mM glucose-saline containing 10-20 nM $CdCl_2$ the increased MT content of mucosa exerted no effect either on cadmium absorption from the lumen (step I), or on its further transport into the body (step II). Immediately after perfusion, essentially all cadmium removed from the lumen was fully recovered in the intestinal mucosa. About 50% of the mucosal cadmium was found in the sediment after homogenization and centrifugation; a large portion of this cadmium may be assigned to the membrane fraction. The binding of freshly absorbed cadmium in the mucosal cytosol was not restricted to low molecular weight protein, although cadmium binding capacity in the MT fraction of controls as well as of exposed animals greatly exceeded actual binding of newly absorbed cadmium. Our results offer no support for the view that MT in the jejunal mucosa serves as determinant of cadmium absorption.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

EPA-600/J-79-143
Journal Article

Toxicology, 14 (1979) 199—208

ON THE ROLE OF METALLOTHIONEIN IN CADMIUM ABSORPTION BY RAT JEJUNUM IN SITU

DINKO KELLO*, NAOKI SUGAWARA**, CATHLEEN VONER and ERNEST C. FOULKES***

Departments of Environmental Health and Physiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267 (U.S.A.)

(Received September 25th, 1979)
(Accepted November 7th, 1979)

SUMMARY

The rcle of metallothionein (MT) in the mechanism of cadmium absorption from the jejunum was studied in 7—9-week-old-male rats exposed to 50 ppm of cadmium in drinking water for 9 days. Exposed animals contained an average of 144 μg MT/g of mucosal tissue, compared to 40 μg in control animals. During jejunal perfusion in situ with 5 mM glucose-saline containing 10—20 nM $CdCl_2$ the increased MT content of mucosa exerted no effect either on cadmium absorption from the lumen (step I), or on its further transport into the body (step II). Immediately after perfusion, essentially all cadmium removed from the lumen was fully recovered in the intestinal mucosa. About 50% of the mucosal cadmium was found in the sediment after homogenization and centrifugation; a large portion of this cadmium may be assigned to the membrane fraction. The binding of freshly absorbed cadmium in the mucosal cytosol was not restricted to low molecular weight protein, although cadmium binding capacity in the MT fraction of controls as well as of exposed animals greatly exceeded actual binding of newly absorbed cadmium. Our results offer no support for the view that MT in the jejunal mucosa serves as determinant of cadmium absorption.

INTRODUCTION

Sufficient evidence has been accumulated in recent years suggesting that

*Fogarty International Fellow. Institute for Medical Research and Occupational Health, Mose Pijade 158, 41000 Zagreb, Yugoslavia.
**Department of Public Health, Sapporo Medical College, S-1 W-17, Sapporo, Japan 060.
***To whom correspondence and reprint requests should be addressed.
Abbreviation: MT, metallothionein.

oral intake represents the primary source of the cadmium accumulated in the human body [1—4]. Although cadmium absorption from the gastrointestinal tract has been investigated in a number of studies, the exact mechanism of cadmium transport from the intestinal lumen to blood is still unknown [3,5,6].

It has been suggested that MT, the low molecular weight Cd-binding protein found in mucosa [7—10], plays an active role in cadmium absorption from the gastrointestinal tract [6,11]. Although it is generally believed that intestinal MT is capable of sequestering intracellular cadmium, thus preventing transfer of the metal to the basolateral membrane, evidence for such a conclusion remains incomplete. It has even been proposed that the intestinal absorption of cadmium might possibly be increased in animals pretreated with cadmium [12].

The purpose of the present experiments was to study the role of mucosal MT in cadmium transport by rat intestine. Both uptake of cadmium from the lumen [13] (step I), and its further movement into the body (step II) were measured in animals as a function of intestinal MT levels. Results obtained permit the conclusion to be drawn that under present experimental conditions, MT does not influence Cd transport by rat jejunum.

MATERIALS AND METHODS

Animals and diets

Male rats of the Sprague—Dawley strain aged 7—9 weeks were obtained from Charles River Co. and maintained on Purina rat chow. This commercial diet contained an average of 0.11 ppm of cadmium, 65 ppm of zinc and 355 ppm of iron (supplier's analysis). Half the animals were exposed for 9 days to 50 ppm cadmium (as $CdCl_2$) in deionized water, offered ad lib; control animals received regular tap water.

Intestinal uptake and transport of cadmium

The rats were fasted for 24 h prior to the experiment. Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (50 mg/kg). The abdomen was opened by a midline incision, and the proximal jejunum was identified, starting at the ligament of Treitz. A segment approx. 15 cm in length was selected and ligatures were loosely applied proximally and distally, care being taken not to compromise the mesenteric circulation to the bowel. Inflow and outflow catheters were inserted and sutures were tightened; the abdominal incision was closed with surgical clamps. Body temperature was maintained with a heating pad and was monitored with a rectal thermometer. The lumen of the jejunal segment was perfused with 10 ml of solution (saline with 5 mM glucose) containing 200 nmol cadmium chloride labeled with 0.5 μCi ^{109}Cd (New England Nuclear, Boston, MA), at a flow rate of 0.4 ml/min for 25 min. The concentration of Cd of 0.02 mM is lower than that used by many other investigators. It was chosen because it does not saturate the mechanism responsible for Cd transport at levels which might be encountered in polluted environments [13]. Immediately

following the perfusion, the lumen was rapidly flushed with 10 ml of ice-cold 0.15 M NaCl followed by 10 ml of air. Blood (6—8 ml) was collected from the abdominal aorta. The jejunal segment, liver and kidneys were removed, weighed and their radioactivity counted in an automatic well-type scintillation counter (Packard, Model A-5921). Mucosa was scraped from the serosal layers of the entire length of perfused jejunal segment with a glass slide, weighed, counted, and stored at −65°C. The ^{109}Cd activity of the remaining carcass of each rat was determined in a whole-body counter using a 5 × 5 inch NaI (TL) crystal connected to a Packard analyzer (Packard, Model A-5921). Results are expressed as nmol $CdCl_2$, as calculated from the specific activity of the solution.

Rate of transmural transport of cadmium

Because the amount of Cd taken up by the jejunum very greatly exceeds that further transported into the body, i.e. because the rate of step I greatly exceeds that of step II, accurate comparison of the 2 steps and an appropriate mass-balance of transported Cd could not be readily achieved with the whole body counter. A technique was therefore developed [14] for measurement of steps I and II on the basis of mass-balance measurements. In a manner similar to that previously described [13], a small volume (1.6—2 ml) of the same solution as above was recirculated through the jejunal lumen at 0.8 ml/min; perfusion pressure did not exceed 2 cm H_2O. The rate of step I was computed from the fall in the ratio of $^{109}Cd/^{3}H$, as determined with a Packard liquid scintillation spectrometer. At the end of perfusion remaining perfusate was collected and combined with 3 ml saline used to wash out the lumen. Quantity A, the amount of Cd removed from the lumen, was calculated from the total ^{109}Cd content of this solution, as measured on a Packard well-type scintillation spectrometer. If, immediately after washing, the intestine was cut out and its ^{109}Cd content (quantity B) determined, essentially all Cd removed from the lumen could be recovered in the tissue (Fig. 1). To determine step II, the catheters were clamped and the abdominal incision closed. Two hundred and seventy-five minutes later, the segment of jejunum was removed for determination of its radioactivity, together with that of its contents. At this time, recovery from the tissue fell below the amount originally absorbed by the tissue, i.e. $A > B$. The deficit in recovery (C), where $A - B = C$, provides a measure of step II. After it had been blotted on the outside, the tissue was then weighed, so that the rates of steps I and II could be calculated in nanomoles/g fresh wt/min.

Cadmium binding components in perfused jejunal mucosa

To determine which tissue components react with freshly absorbed cadmium, mucosa obtained from perfused jejunal segments was homogenized in 0.25 M sucrose (1 : 4 w/v) and centrifuged at 100 000 *g* for 1 h. The radioactivity in supernatant and precipitate was determined. The supernatant was chromatographed on a Sephadex G-75 column (1.5 × 30 cm) in 20 mM Tris—HCl buffer, pH 8.6 and the radioactivity of each fraction was

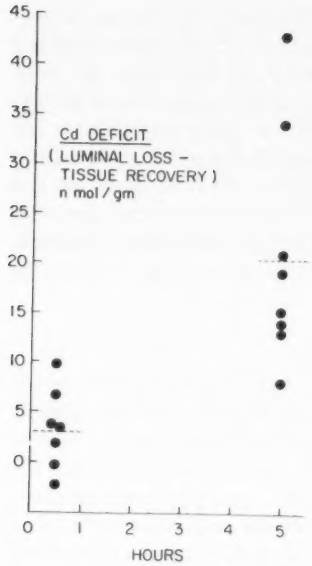


Fig. 1. Quantitation of step II by measurement of the difference (C) between Cd removed from the lumen during 25 min perfusion (A) and that recovered from the intestinal wall at the times shown (B), i.e. C = A − B. Time is measured from the beginning of perfusion, and mean deficits are indicated by dashed lines.

measured. The results are expressed as percent of total ^{109}Cd radioactivity in homogenate.

Determination of intestinal metallothionein

The total amount of MT in the mucosa was determined with the saturation method described by Kotsonis and Klaassen [15]. Homogenized mucosa (25% in 0.25 M sucrose) was centrifuged at 10 000 *g* for 15 min, 10% TCA was added to the supernatant to a final pH of 2. The mixture was centrifuged again at 15 000 *g* for 30 min. The TCA supernatant was mixed with cadmium solution (300 nmol $^{109}CdCl_2$/g tissue). The pH was adjusted to 8.6 with dilute NaOH and the solution was fractionated on a Sephadex G-75 column (1.5 × 30 cm) and the radioactivity of each fraction was determined. The amount of cadmium in the MT peak was calculated from the specific activity of the $^{109}CdCl_2$. The amount of Cd-binding protein of low molecular weight was then derived from its Cd-binding capacity on the assumption that it binds 7 mol Cd/mol of 10 000.

Determination of cadmium and zinc

The intestine was thoroughly washed with saline before the analysis. Cadmium and zinc were determined in mucosa by atomic absorption spectro-

photometry (Perkin Elmer, Model 403) after wet digestion with nitric and perchloric acid. In samples from control group of animals cadmium was extracted and concentrated using the method described by Yeager et al. [16].

Statistics

All results are expressed as the arithmetic mean with the standard deviation of the mean. The significance of the difference among the groups was determined by Student's *t*-test for unpaired data.

RESULTS

Induction of metallothionein in intestinal mucosa

The daily intake of cadmium in animals exposed to 50 ppm cadmium in drinking water has been estimated as 1.4 mg, i.e. total intake of cadmium after 9 days was about 12.6 mg. Data presented in Table I show that such treatment caused an increase in the amount of cadmium and metallothionein in mucosa. Mucosal cadmium in pretreated rats was 68 times higher than in control animals; at the same time the concentration of metallothionein increased only 3.6 times. The concentration of zinc in these 2 groups of animals did not differ significantly.

Intestinal uptake and transport of cadmium

The data presented in Table II demonstrate that after intestinal perfusion with 0.02 mM $^{109}CdCl_2$ solution for 25 min, extensive accumulation of cadmium was found only in the intestinal wall. By contrast, the total intestinal transfer of cadmium, expressed as the sum of cadmium recovered in carcass, liver, kidneys and blood, represented less than 0.3 percent of the total perfused dose in both groups of animals. As further described below essentially all cadmium accumulated by the intestine was confined to the mucosa.

TABLE I

CONCENTRATIONS OF CADMIUM, ZINC AND METALLOTHIONEIN IN MUCOSA (μg/g wet wt)

Results are expressed as arithmetic mean ± S.D. Exposed animals had 50 ppm of cadmium in drinking water for 9 days.

	6 Control rats	6 Pretreated rats
Cadmium	0.06 ± 0.04	4.32 ± 0.86
Zinc	21.5 ± 3.4	22.2 ± 4.1
Cd-binding capacity[a]	3.2 ± 0.5	11.3 ± 2.8
Metallothionein	40 ± 6	144 ± 36

[a]Equivalent to total amount of Cd bound in MT fraction, as measured during MT assay.

Although these results (Table II) show that the intestinal uptake of cadmium was slightly higher in pretreated than in control animals (17% and 13% of dose respectively, $P < 0.05$), the total transmural transport was the same in both groups.

Cadmium binding components in perfused jejunal mucosa

In order to determine the distribution of cadmium in the intestinal wall, the mucosa of perfused jejunal segments was harvested by scraping and analyzed. The supernatant fraction of mucosal homogenates in both groups of animals contained about one-half of total tissue ^{109}Cd (Table III). Although the supernatant from exposed animals contained a little more cadmium than that from controls, major differences between these 2 groups appeared after chromatography. As shown in Table III there were 2 major cadmium peaks in both groups. The second peak corresponds to a mol. wt of about 10 000; the first peak contains high molecular weight compounds. In control rats most of the ^{109}Cd appeared near the void volume, indicating that most of the cadmium was bound to high molecular weight proteins and very little to MT. In contrast, in the pretreated rats little cadmium was bound to high molecular weight proteins and more was associated with the low molecular weight fraction, tentatively identified as MT. In neither group did significant amounts of cadmium remain unbound.

Rate of transmural transport of cadmium

Because of the small amount of Cd transferred to the body during 25 min perfusion (Table II), the transfer step (step II) was measured over a period of 5 h as discussed in the Materials and Methods section. Table IV, based on results similar to those shown in Fig. 1, demonstrates that the rate of trans-

TABLE II

INTESTINAL UPTAKE AND TRANSMURAL TRANSPORT OF CADMIUM

Perfusate contained 200 nmol $CdCl_2$. Results are expressed as nmoles of cadmium (Mean ± S.D.). Rats were killed for tissue analyses after 25 min perfusion.

	15 Control rats	15 Pretreated rats
Weight of intestine (g)	1.4 ± 0.3	1.4 ± 0.2
Intestinal uptake	25.1 ± 6.5	34.0 ± 13.3
Retained in mucosa	24.5 ± 6.4	33.1 ± 12.8
Carcass	0.211 ± 0.091	0.277 ± 0.192
Liver	0.186 ± 0.108	0.252 ± 0.180
Kidney	0.007 ± 0.003	0.012 ± 0.006
Blood	0.007 ± 0.003	0.016 ± 0.009
Total transfer to body	0.412 ± 0.136	0.555 ± 0.319

TABLE III

DISTRIBUTION OF PERFUSED CADMIUM IN JEJUNAL MUCOSA

Results are expressed as percent of levels in original homogenate.

	5 Control rats	5 Pretreated rats
Homogenate	100.0	100.0
Sediment	51.4 ± 2.5	40.4 ± 5.7
Supernatant	48.6 ± 2.5	59.6 ± 5.7[a]
High mol. wt fraction (above 10 000)	30.0 ± 3.7	17.2 ± 1.8[b]
Low mol. wt fraction (approx. 10 000)	13.4 ± 5.1	38.9 ± 7.2[b]
Remaining fraction (below 10 000)	5.6 ± 1.0	3.4 ± 0.5

[a] $P < 0.01$.
[b] $P < 0.001$.

mural transport as measured by disappearance of cadmium over a period of 5 h was low and did not exceed 1.3% of the rate of cadmium removal from the lumen. The ratio of step II to step I was the same in both groups of animals.

DISCUSSION

While relatively little is known about the mechanism of cadmium absorption, it is clear that this process must consist of at least 2 steps: (1) uptake

TABLE IV

RATE OF UPTAKE AND TRANSMURAL TRANSPORT OF CADMIUM

Results are shown as mean ± S.D.

	10 Control rats	13 Pretreated rats
Intestinal uptake[a] (nmol/g/min)	1.9 ± 0.8	1.3 ± 0.4
Transmural transport[b] (nmol/g/min)	0.023 ± 0.010	0.015 ± 0.009
Rate of transmural transport as % of rate of uptake	1.3 ± 0.7	1.3 ± 0.8

[a] Jejunal segment was perfused with 0.02 mM $^{109}CdCl_2$ solution for 25 min.
[b] Rate of transport was calculated from difference between initial uptake and final retention of ^{109}Cd in tissue 275 min after the end of perfusion.

of the metal by the mucosal cells; and (2) transmural movement of cadmium into the body [6,14,17]. Despite differences in methodology and species, there is considerable agreement between the results presented here and those obtained by previous workers [5,6,17], namely that intestinal uptake of cadmium is much higher than its transport into the body (Table II). Thus, we found that even after 5 h, only a small portion of cadmium accumulated in jejunal mucosa is released into the animal; the rate of release amounted to only 1–2% of the rate of uptake (Table IV).

It is important to emphasize that under present conditions, in the absence of normal luminal contents, uptake of cadmium in all likelihood greatly exceeds that to be expected in presence of normal intestinal contents. We may assume that in rats on a normal diet most of the metal is bound to non-absorbable food components and is therefore eliminated from the body [18]; there is evidence also for the presence in food of direct inhibitors of cadmium absorption [3].

The prolonged retention of cadmium by the intestinal tract was attributed to extracellular adsorption [17,19] or intracellular accumulation and binding on different ligands, particularly MTs [12,20]. Sahagian et al. [17] proposed that in the process of mucosal uptake, cadmium probably reacts with the cell membrane. This is fully compatible with the present study which shows that about one-half of ^{109}Cd present in mucosal homogenate is collected in the precipitate after centrifugation. Since numerous studies have shown that only a few percent of intracellular cadmium are bound to nuclei, mitochondria and endoplasmic reticulum [21], a large portion of the cadmium in the precipitate may be assigned to the membrane fraction. Similar findings by Taguchi and Suzuki [22] support this conclusion. Detailed information about the nature of binding sites or classes of ligands present in membranes for a given metal is not available.

Recently several authors suggested that intestinal MT plays an important role in cadmium absorption from the gastrointestinal tract [6,10,11]. It was postulated that this protein would sequester intracellular cadmium and prevent in this way its transport into the circulation [12,20]. This statement is based on the assumption that the sequestering of cadmium in the mucosa resembles the handling of iron [20] which, when taken up by the mucosa in excess of bodily needs, is believed to bind to ferritin and subsequently to be excreted upon desquamation of the epithelium. This hypothesis, though attractive, is not fully supported by available evidence. Thus, Sasser and Jarboe [19] and Foulkes and Stemmer (unpublished observation) observed accumulation of cadmium in the deeper mucosal layers which do not readily participate in the rapid turnover of epithelial cells. In addition, the binding of cadmium observed in the present study was pronounced even in control animals, although these contained only small levels of MT in the mucosa. Clearly, binding of cadmium is not restricted to MT, a conclusion which raises questions about the proposed primary role of this compound in the sequestration of the metal.

A similar conclusion can be drawn from the fact that the great increase

in MT seen in exposed animals was not accompanied by either significant increases in cadmium uptake or increases in its retention in the mucosa. Indeed, as can be deduced from Table I, an increase in the Cd-binding capacity of the MT fraction from 28 to 100 nmol/g mucosa exerted no effect on the transmural transport of cadmium (Tables II and IV). Even a partial involvement of the relatively very large Cd-binding capacity in the mucosal MT fraction should have been reflected in some change in the very much smaller amounts of cadmium transported across the tissue. The small increase in accumulation of ^{109}Cd in mucosa of pretreated rats (Table II) may perhaps be explained by alterations in the permeability of the membranes [20,23].

Different authors found that cadmium in the cytosol of intestinal tissue is mainly bound to MT [7—10]. We observed that cadmium, freshly absorbed by control animals, reacts primarily with high molecular weight proteins rather than with MT (Table III), although the mucosa actually contained significant amounts of the protein (Table I). Presence of MT in normal animals has previously been reported by other authors [8,20], and presumably reflects normal background levels induced by zinc in the diet. Our calculations show that cadmium binding capacity of MT, even in control animals (Table I), greatly exceeds actual binding of newly absorbed cadmium (Tables II and III). Kotsonis and Klaassen [11] recently reported similar findings in experiments involving measurement of Cd-binding capacity of MT in liver and kidneys. Apparently, even though MT may not be fully saturated with metal, a variety of ligands can and do compete for freshly absorbed cadmium. Obviously, a short segment of jejunum in the surgically prepared animal is not fully representative of the intestine in an intact rat. Nevertheless, our results clearly show that MT in the mucosa does not serve as a primary determinant of cadmium absorption, at least under present experimental conditions.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grant ES-01462 and by US EPA grant R-805840010. The authors are grateful to Mrs. Sheila Blanck and Mrs. Mirjana Kello for their valuable technical assistance.

REFERENCES

1 L. Friberg, M. Piscator, G.F. Nordberg and T. Kjellstrom, Cadmium in the Environment, CRC Press, Cleveland, Ohio, 1974.
2 G.F. Nordberg, Effects and Dose-Response Relationship of Toxic Metals, Elsevier, Amsterdam, 1976.
3 I. Bremner, Cadmium toxicity, in G.H. Bourne, (Ed.), Human and Animal Nutrition. World Review of Nutrition and Dietetics, Vo. 32, S. Karger Publ., Basel, 1978, p. 165.
4 B.A. Fowler, Environ. Health Perspect., 28 (1979) 297.
5 S.I. Koo, C.S. Fullmer and R.H. Wasserman, J. Nutr., 108 (1978) 1812.
6 J. McGivern and J. Mason, J. Comp. Pathol., 89 (1979) 293.
7 K. Tanaka, K. Nishiguchi and K. Okahara, J. Hyg. Chem., 19 (1973) 202.

8 R.W. Chen and H.E. Ganther, Environ. Physiol. Biochem., 5 (1975) 378.
9 C. Sugawara and N. Sugawara, Bull. Environ. Contam. Toxicol., 14 (1975) 159.
10 M.G. Cherian, R.A. Goyer and L.S. Valberg, J. Toxicol. Environ. Health, 4 (1978) 861.
11 F.N. Kotsonis and C.D. Klaassen, Toxicol. Appl. Pharmacol., 46 (1978) 39.
12 K.S. Squibb, R.J. Cousins, B.L. Silbon and S. Levin, Exp. Mol. Pathol., 25 (1976) 163.
13 E.C. Foulkes, J. Environ. Pathol. Toxicol., 1979, in press.
14 E.C. Foulkes and C. Voner, Fed. Proc. 1980, in press.
15 F.N. Kotsonis and C.D. Klaassen, Toxicol. Appl. Pharmacol., 42 (1977) 583.
16 D.W. Yeager, J. Cholak and E.W. Henderson, Environ. Sci. Technol., 5 (1971) 1020.
17 B.M. Sahagian, I. Harding-Barlow and H.M. Perry Jr., J. Nutr., 93 (1967) 291.
18 D. Kello and K. Kostial, Toxicol. Appl. Pharmacol., 40 (1977) 277.
19 L.B. Sasser and G.E. Jarboe, Toxicol. Appl. Pharmacol., 41 (1977) 423.
20 L.S. Valberg, J. Sorbie and D.L. Hamilton, Am. J. Physiol., 231 (1976) 462.
21 D. Bhattacharjee, T.K. Shetty and K. Sundaram, Indian J. Exp. Biol., 17 (1979) 74.
22 T. Taguchi and S. Suzuki, Jpn. J. Hyg., 33 (1978) 467.
23 L.S. Valberg, J. Haist, M.G. Cherian, L. Delaquerriere-Richardson and R.A. Goyer, J. Toxicol. Environ. Health, 2 (1977) 963.